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(54) Title: GLYCOGEN SYNTHASE KINASE-3 INHIBITORS

(57) Abstract: The invention is directed to a highly effective and specific peptide inhibitors of glycogen synthase kinase-3 (GSK-3) and useful implications of these peptides. The peptide inhibitors of the invention include therewithin the amino acid motif XZXXXS (p)X, where S(p)=phosphorylated serine or phosphorylated threonine, X=any amino acid, and Z=any amino acid except serine, or threonine. The peptides competitively bind to GSK-3 in vitro with high affinity. Because the amino acid Z in the motif is not phosphorylated, the peptide inhibitor cannot be phosphorylated. Thus, the peptide inhibits the catalytic activity of GSK-3, since the enzyme cannot proceed to phosphorylate other proteins.

GLYCOGEN SYNTHASE KINASE-3 INHIBITORSTECHNICAL FIELD OF THE INVENTION

This invention relates generally to novel peptide inhibitors of glycogen synthase kinase-3 and the use thereof for regulating biological conditions mediated by GSK-3 activity. The invention particularly relates to the use of such inhibitors to potentiate insulin signaling in type-2 diabetics.

BACKGROUND OF THE INVENTION

The regulation of glycogen catabolism in cells is a critical biological function that involves a complex array of signaling elements, including the hormone insulin. Through a variety of mediators, insulin exerts its regulatory effect by increasing the synthesis of glycogen by glycogen synthase (GS). A key event in insulin action is the phosphorylation of insulin receptor substrates (IRS-1, IRS-2) on multiple-tyrosine residues, which results in simultaneous activation of several signaling components, including PI3 kinase (Myers et al, 1992)). Similarly, the activity of glycogen synthase is suppressed by its phosphorylation.

One of the earliest changes associated with the onset of type 2 (non-insulin dependent) diabetes is insulin resistance. Insulin resistance is characterized by hyperinsulemia and hyperglycemia. Although the precise molecular mechanism underlying insulin resistance is unknown, defects in downstream components of the insulin signaling pathway may be the cause. Among the downstream components of insulin signaling is glycogen synthase kinase-3 (GSK-3), a serine/threonine kinase that has recently been

recognized as an important signaling molecule in a variety of cellular processes. High activity of GSK-3 impairs insulin action in intact cells (Eldar-Finkelman et al, 1997). This impairment results from the phosphorylation of 5 insulin receptor substrate-1 (IRS-1) serine residues by GSK-3. Likewise, increased GSK-3 activity expressed in cells results in suppression of glycogen synthase activity (Eldar-Finkelman et al, 1996). The laboratory of the present inventor found that GSK-3 activity is significantly 10 increased in epididymal fat tissue of diabetic mice (Eldar-Finkelman et al, 1999). Subsequently, increased GSK-3 activity was detected in skeletal muscle of type 2 diabetes patients (Nickouline et al, 2000). Thus, the inhibition of 15 GSK-3 activity may represent a way to increase insulin activity *in vivo*.

The inhibition of GSK-3 by lithium chloride (LiCl) (PCT International patent application WO 97/41854) and by purine inhibitors (PCT International patent application WO 98/16528) has been reported. However, these inhibitors are 20 not specific for GSK-3. Similarly, an engineered cAMP response element binding protein (CREB), a known substrate of GSK-3, has been described (Fiol et al, 1994), as have two peptide inhibitors of GSK-3 (Fiol et al, 1990). However, these substrates only nominally inhibit GSK-3 activity.

Recent work has demonstrated that GSK-3 is 25 involved in additional cellular processes including development (He et al, 1995), oncogenesis (Rubinfeld et al, 1996) and protein synthesis (Welsh et al, 1993). Importantly, GSK-3 plays a negative role in these pathways. 30 This suggests that GSK-3 is a cellular inhibitor in signaling pathways. Thus, development of specific drug inhibitors for GSK-3 will have important implications in basic research, as well as therapeutic interventions.

Other activities of GSK-3 in a biological context include GSK-3's ability to phosphorylate tau protein *in vitro* as described in Mandelkow et al (1993), Mulot et al (1994), and Lovestone et al (1995) and in tissue culture 5 cells as described in Latimer et al (1995). Phosphorylation of tau and polymerization of the phosphorylated tau is believed to allow formation of paired helical filaments that are characteristic of Alzheimer's Disease. Thus, inhibition of GSK-3 may be useful to treat or inhibit these disorders.

10 Thus, a need remains in the art for small, highly-specific, highly-effective peptide inhibitors of GSK-3. Such inhibitors would be useful in treating conditions associated with elevated GSK-3 activity such as diabetes type 2 (Eldar-Finkelman et al, 1997; Nikouolina et al, 2000), 15 Alzheimer's Disease (Mulot et al, 1995) and manic depression (Manji et al, 1999).

SUMMARY OF THE INVENTION

The invention is directed to a highly effective and specific peptide inhibitors of glycogen synthase kinase-20 3 (GSK-3) and useful implications of these peptides. The peptide inhibitors of the invention include therewithin the amino acid motif XZXXXS(p)X, where S(p)=phosphorylated serine or phosphorylated threonine, X=any amino acid, and Z=any amino acid except serine, or threonine. The peptides 25 competitively bind to GSK-3 *in vitro* with high affinity. Because the amino acid Z in the motif is not phosphorylated, the peptide inhibitor cannot be phosphorylated. Thus, the peptide inhibits the catalytic activity of GSK-3, since the enzyme cannot proceed to phosphorylate other proteins.

30 The invention is based on the idea that short peptides derived from the recognition motif of GSK-3 may serve as inhibitors of the enzyme. The recognition motif of

GSK-3 is SXXXS(p) where S=serine or threonine, X=any amino acid and S(p)= phosphorylated serine or phosphorylated threonine. This motif is unique to GSK-3. Because other protein kinases will not compete for this peptide, the GSK-3 inhibition motif is a specific and selective inhibitor for GSK-3.

The present invention presents a rationale and strategy to develop peptide inhibitors. The peptide inhibitors of the invention can be used to inhibit the activity of GSK-3 or to potentiate insulin signaling *in vivo*. The peptide inhibitors are useful for treating type 2 diabetes in a patient or preventing type 2 diabetes in a subject, as well as in identifying inhibitors of GSK-3. The peptide inhibitors are also useful therapeutic or research tools in the areas of oncogenesis, development, and metabolism, where GSK-3 has been shown to be important, as well as in the treatment of Alzheimer's Disease.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing inhibition of GSK-3 by peptide inhibitor. Purified GSK-3 enzyme was incubated with indicated concentrations of peptide #5 together with peptide substrate (PGS-1 (Manji et al, 1999)) and ^{32}P labeled ATP. The reactions were incubated at 30°C for 15 minutes. Reactions were spotted on p81 (phosphocellulose papers) washed with phosphoric acid and counted for radioactivity. Results are presented as ^{32}P incorporation into peptide.

Figures 2A-2B indicate that peptide inhibitors are competitive inhibitors. Purified GSK-3 was incubated with 200 μM peptides inhibitors together with PGS-1 substrate and ^{32}P labeled ATP. The reactions were incubated at 30°C for 15 minutes. Reactions were spotted on p81 (phosphocellulose papers) washed with phosphoric acid and counted for

radioactivity. Results are presented as ^{32}P incorporation into peptide (Figure 2A). Lineweaver-Burk plots of data are shown in Figure 2B. Filled circles - no peptide, hollow circles - peptide #5, hollow triangles - peptide #7, filled 5 triangles - peptide #8.

Figure 3 shows the effect of peptide inhibitor on glycogen synthase activity in intact cells. 293 cells were treated with HP9c for 2.5 hours. Cells were lysed and GS activity was determined as described (Eldar-Finkelman et al, 10 1996). Results present fold stimulation of GS activity observed in control cells treated with DMSO vehicle. Data are mean \pm SE of 3 independent experiments.

Figure 4 shows that peptide inhibitor HP9c enhances allosteric activation of glycogen synthase by G6P. 15 293 cells were treated with 60 μM HP9c for 2.5 hours. Glycogen synthase activity was assayed in cell extracts in the presence of varied concentration of G6P as indicated. G6P dose response curve of one representative experiment is presented. Each point represents an average of duplicated 20 samples. Filled circles - control. Hollow circles - peptide treated cells.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the concept that relatively short peptides, derived from the recognition 25 motif of GSK-3, may serve as enzyme inhibitors. The recognition motif of GSK-3 is SXXXS(p), where S=serine or threonine, X=any amino acid, and S(p)=phosphorylated serine or phosphorylated threonine. This recognition motif is unique to GSK-3. In experiments designed to discover 30 substrate inhibitors of GSK-3, it has been determined that the phosphorylated serine or threonine residue is necessary for binding. Without this residue, the peptide will neither

be a substrate nor an inhibitor. In order to be a substrate, there must be a serine (or threonine) residue upstream of the phosphorylated serine (or threonine) residue separated by three additional residues. It has now been
5 determined that replacing this upstream serine or threonine from the substrate recognition motif with a residue other than serine or threonine, the substrate peptide is converted from a substrate to an inhibitor. It is believed that at least one additional residue upstream and downstream of this
10 motif is necessary in order to have an inhibitor of minimum size. Thus, the recognition motif which is believed to be necessary in order for the peptide to serve as an inhibitor is XZXXXS(p)X, where S(p)=phosphorylated serine or threonine, X is any amino acid, and Z is any amino acid
15 except serine or threonine.

The preferred peptides in accordance with the present invention maintain the sequence of a known substrate except for the substitution of the serine or threonine that is at the fourth position upstream of the phosphorylated serine or threonine. When the known substrate from which the inhibitor is derived is the CREB protein, the minimum size of the inhibitor peptide is 10 residues, with the additional three residues all being upstream of the Z. Similarly, when the substrate from which the inhibitor is derived is HSF-1, the minimum number of residues in the inhibitor peptide must be greater than seven. It is known that a peptide of 11 residues is a strong inhibitor.
20 Routine experimentation will determine whether peptides of 8, 9 and 10 residues inhibit or not. The present invention
25 only comprehends the peptides within the formula of the present invention that are active as inhibitors.
30

Previously, Fiol et al (1990) experimented with glycogen synthase as a substrate of GSK-3 and determined

that a peptide fragment, where the serine at the fourth position upstream of the phosphorylated serine in the recognition motif is substituted by alanine, has only very weak inhibiting activity. The present inventor has
5 concluded that substrates which contain multiple recognition motifs behave differently from substrates which have only a single such recognition motif. Accordingly, the peptide inhibitors of the present invention will not comprehend peptides which contain multiple recognition motifs in the 20
10 residues upstream of the inhibition motif. Thus, the present invention specifically excludes peptides having two or more recognition motifs therein upstream of the inhibition motif. The preferred embodiment of the present invention are peptides having no recognition motifs.
15 Indeed, the most preferred embodiments of the present invention are the shortest peptides having the inhibition motif of the present invention and which exhibit inhibition activity.

Thus, the present invention is directed to a novel
20 class of peptide inhibitors of GSK-3, which exhibit high specificity for GSK-3 and strongly inhibit it with an IC₅₀ of about 150 µM, measured by *in vitro* kinase assay (see Example 2). The peptide inhibitors are "pseudosubstrates," because they have been derived and modified from the SXXXS(p)
25 recognition motif of GSK-3. This strategy was exemplified by using two prototype peptides derived from CREB or HSF-1 proteins. One of the known substrates of GSK-3 is cAMP response element binding protein (CREB). The CREB sequence encompassing the GSK-3 recognition motif has been
30 identified. CREB is phosphorylated at a single serine residue, Ser₁₃₃, to create the sequence motif SXXXS(p), where S(p) denotes phosphorylated serine, which serves as the

minimal GSK-3 recognition sequence (Fiol et al, 1987; Fiol et al, 1988).

The peptides are small, synthetic peptides of about 7 to 50 amino acids and have as part of their sequence 5 the amino acid motif XZXXXS(p)X, where S(p)=phosphorylated serine or threonine, X=any amino acid, and Z=any amino acid except serine or threonine. Because the amino acid Z in the motif is not phosphorylated, the peptide inhibitor cannot be phosphorylated. Thus, the peptide inhibits the catalytic 10 activity of GSK-3 because the enzyme cannot proceed to phosphorylate other proteins.

Peptide inhibitors of the present invention inhibit both GSK-3 substrate phosphorylation and autophosphorylation on a level comparable to LiCl (see 15 EXAMPLE 2), as measured by *in vitro* kinase assay.

Peptide inhibitors of about 7-10 amino acid residues or greater are sufficient to inhibit GSK-3 activity. The peptide inhibitors can be about 10-13 amino acid residues in length. Also, the peptide inhibitors can 20 be about 7-50 amino acids in length. Inhibitors of 7-20 amino acid residues in length are preferred, with a length of 10-20 amino acids being more preferred, and 10-13 amino acids most preferred.

Most of the GSK-3 substrates which do not contain 25 multiple recognition motifs have a glutamic acid residue three residues upstream of the first serine residue of the recognition motif. When this glutamic acid is replaced by another amino acid residue, the inhibiting activity of the peptides of the present invention are further improved. 30 Accordingly, a further preferred embodiment of the present invention are peptides in which the residue three residues upstream of Z is other than glutamic acid.

The inhibition of GSK-3 activity is a way to increase insulin activity *in vivo*. High activity of GSK-3 impairs insulin action in intact cells (Eldar-Finkelman et al, 1997). This impairment results from the phosphorylation 5 of insulin receptor substrate-1 (IRS-1) serine residues by GSK-3. Studies performed in patients with type 2 diabetes (non-insulin dependent diabetes mellitus, NIDDM) show that glycogen synthase activity is markedly decreased these patients, and that decreased activation of protein kinase B 10 (PKB), an upstream regulator of GSK-3, by insulin is also detected (Shulman et al, (1990); Nikouline et al, (1997); Cross et al, (1995). Mice susceptible to high fat diet-induced diabetes and obesity have significantly increased GSK-3 activity in epididymal fat tissue (Eldar-Finkelman et 15 al, 1999). Increased GSK-3 activity is expressed in cells resulted in suppression of glycogen synthase activity (Eldar-Finkelman et al, 1996). Thus, inhibition of GSK-3 activity provides a useful method for increasing insulin activity in insulin-dependent conditions.

20 An inhibitor that "potentiates insulin signaling" is an inhibitor which, when administered, increases the phosphorylation of insulin receptor downstream components and increases the rate of glucose uptake as compared to glucose uptake in a subject not administered the inhibitor.

25 The term "treatment" as used herein refers to reducing or alleviating symptoms in a subject, preventing symptoms from worsening or progressing, inhibition or elimination of the causative agent, or prevention of the infection or disorder in a subject who is free therefrom.
30 Thus, for example, treatment of type 2 diabetes means alleviating, ameliorating, inhibiting, reducing, or curing the clinical manifestations of type 2 diabetes, either transiently or permanently, including slowing the rate of

glucose uptake. Such treatment includes the potentiation of insulin signaling. The "preventing" of type 2 diabetes means inhibiting, delaying, slowing, or preventing the onset of clinical manifestations of type 2 diabetes, either
5 transiently or permanently, including slow rate of glucose uptake. Such prevention includes potentiation of insulin signaling. An "amount effective to potentiate insulin signaling" is the dose of inhibitor needed to effectively potentiate insulin signaling in a subject. Treatment of
10 Alzheimer's disease may be halting or retarding the progression of the disease (e.g., as measured by a reduction in the rate of dementia).

The term "biological condition mediated by GSK3 activity" as used herein refers to any biological or medical
15 condition or disorder in which effective GSK3 activity is identified, whether at normal or abnormal levels. The condition or disorder may be caused by the GSK3 activity or may simply be characterized by GSK3 activity. That the condition is mediated by GSK3 activity means that some
20 aspect of the condition can be traced to the GSK3 activity. It is expected that by the method of the invention, inhibiting the GSK3 activity will then prevent, ameliorate or treat the condition so characterized.

Method of Making Peptide Inhibitors. The amino acid residues described herein are preferably in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the peptide inhibitors retain specificity for GSK-3. This definition includes, unless otherwise specifically
25 indicated, chemically-modified amino acids, including amino acid analogs (such as penicillamine, 3-mercaptop-D-valine), naturally-occurring non-proteogenic amino acids (such as norleucine), and chemically-synthesized compounds that have

properties known in the art to be characteristic of an amino acid. The term "proteogenic" indicates that the amino acid can be incorporated into a protein in a cell through well-known metabolic pathways.

5 In other words, the peptide can be a peptide "mimetic". Thus, one aspect of the present invention provides for peptidomimetics which mimic the structural features of the critical amino acid motif XZXXXS(p)X.

10 Although most inhibitors of GSK-3 are expected to be peptides, by the use of the screening method described below, other non-peptide inhibitors of GSK-3 can be identified. The peptidomimetics that are non-peptide in nature can be designed and synthesized by standard organic chemical methods. The peptidomimetics that are non-peptide 15 in nature can be even more advantageous in therapeutic use, in the resistance to degradation, in permeability and in possible oral administration.

20 Peptidomimetics are small molecules that can bind to proteins by mimicking certain structural aspects of peptides and proteins. They are used extensively in science and medicine as agonists and antagonists of protein and peptide ligands of cellular and other receptors, and as substrates and substrate analogs for enzymes. Some examples 25 are morphine alkaloids (naturally-occurring endorphin analogs), penicillins (semi-synthetic), and HIV protease inhibitors (synthetic). Such compounds have structural features that mimic a peptide or a protein and as such are recognized and bound by other proteins. Binding the peptidomimetic either induces the binding protein to carry 30 out the normal function caused by such binding (agonist) or disrupts such function (antagonist, inhibitor).

A primary goal in the design of peptide mimetics has been to reduce the susceptibility of mimetics to

cleavage and inactivation by peptidases. In one approach, such as disclosed by Sherman et al (1990), one or more amide bonds have been replaced in an essentially isosteric manner by a variety of chemical functional groups. This stepwise 5 approach has met with some success in that active analogs have been obtained. In some instances, these analogs have been shown to possess longer biological half-lives than their naturally-occurring counterparts

In another approach, a variety of uncoded or 10 modified amino acids such as D-amino acids and N-methyl amino acids have been used to modify mammalian peptides. Alternatively, a presumed bioactive conformation has been stabilized by a covalent modification, such as cyclization or by incorporation of γ -lactam or other types of bridges. 15 See, e.g., Veber et al, (1978) and Thorsett et al (1983).

Another approach, disclosed by Rich (1986), has been to design peptide mimics through the application of the transition state analog concept in enzyme inhibitor design. For example, it is known that the secondary alcohol of 20 statine mimics the tetrahedral transition state of the sessile amide bond of the pepsin substrate

Nicolaou et al (1990) disclosed non-peptide somatostatin mimics.

In USP 5,552,534, non-peptide compounds are 25 disclosed which mimic or inhibit the chemical and/or biological activity of a variety of peptides. Such compounds can be produced by appending to certain core species, such as the tetrahydropyranyl ring, chemical functional groups which cause the compounds to be at least 30 partially crossreactive with the peptide. As will be recognized, compounds which mimic or inhibit peptides are to varying degrees crossreactive therewith. Other techniques

for preparing peptidomimetics are disclosed in USP 5,550,251 and USP 5,288,707, for example.

Protein phosphorylation plays a crucial part in the biochemical control of cellular activity.

5 Phosphorylation usually means formation of a phosphate ester bond between a phosphate (PO_4) group and an amino acid containing a hydroxyl (OH) group (tyrosine, serine and threonine). Many phosphorylation sites in proteins act as recognition elements for binding to other proteins, and
10 those binding events activate or deactivate signaling and other pathways. Protein phosphorylation thus acts as a switch to turn biochemical signaling on and off.

15 Phosphopeptide mimetics are a subclass of peptidomimetics that contain analogs of phosphorylated tyrosine, serine and threonine. Phosphate esters may be hydrolyzed by various enzymes, thus turning off a phosphorylation signal. Phosphopeptide mimetics, however, usually contain non-hydrolyzable analogs to prevent
20 inactivation (Burke et al, 1994a; Burke et al, 1996a; Chen et al, 1995; Wiemann et al, 2000; Shapiro et al, 1997; Otaka et al, 1995; Otaka et al, 2000). General examples of phosphopeptide mimetics in the art include SH2 domain
25 analogs (Burke et al, 1994a; Fu et al, 1998; Gao et al, 2000; Mikol et al, 1995; Ye et al, 1995), transcription factor NF-(kappa)B analog (McKinsey et al, 1997), P53 analog (Higashimoto et al, 2000) and protein-tyrosine phosphatase inhibitors (Burke et al, 1994b; Burke et al, 1996b; Groves et al, 1998; Kole et al, 1995; Kole et al, 1997; Roller et al, 1998).

30 Commercially available software packages can be used to design small peptides and/or peptidomimetics containing, phosphoserine or phosphothreonine analogs, preferably non-hydrolyzable analogs, as specific

antagonists/inhibitors. Suitable commercially available software for analyzing crystal structure, designing and optimizing small peptides and peptidomimetics include, but are not limited to: Macromolecular X-ray Crystallography
5 QUANTA Environment (Molecular Simulations, Inc.); TeXsan, BioteX, and SQUASH (Molecular Structure Corporation); and Crystallographica (Oxford Cryostsystems).

The peptide inhibitors of the present invention also include salts and chemical derivatives of the peptides.
10 "Chemical derivative" refers to a polypeptide of the invention having one or more residues chemically derivatized by reaction of a functional side group. Such derivatized molecules include, for example, those molecules in which free amino groups have been derivatized to form amine
15 hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups may be derivatized to
20 form O-acyl or O-alkyl derivatives. Also included as chemical derivatives are those peptides that contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids. For example, 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be
25 substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine; and ornithine may be substituted for lysine. The chemical derivatization does not comprehend changes in functional groups which change one amino acid to another.

30 Some useful modifications are designed to increase the stability of the peptide inhibitor in solution and, therefore, serve to prolong the half-life of the peptide inhibitor in solutions, particularly biological fluids, such

as blood, plasma or serum, by blocking proteolytic activity in the blood. A peptide inhibitor can have a stabilizing group at one or both termini. Typical stabilizing groups include amido, acetyl, benzyl, phenyl, tosyl,
5 alkoxy carbonyl, alkyl carbonyl, benzyloxycarbonyl and the like end group modifications. Additional modifications include using a "L" amino acid in place of a "D" amino acid at the termini, cyclization of the peptide inhibitor, and amide rather than amino or carboxy termini to inhibit
10 exopeptidase activity.

A peptide inhibitor of the invention may or may not be glycosylated. The peptide inhibitors are not glycosylated, for example, when produced directly by peptide synthesis techniques or are produced in a prokaryotic cell
15 transformed with a recombinant polynucleotide. Eukaryotically-produced peptide molecules are typically glycosylated.

The peptide inhibitors of the invention can be produced by well known chemical procedures, such as solution
20 or solid-phase peptide synthesis, or semi-synthesis in solution beginning with protein fragments coupled through conventional solution methods, as described by Dugas et al (1981). Alternatively, a peptide inhibitor of the invention can be synthesized by using well known methods, including
25 recombinant methods and chemical synthesis.

A peptide inhibitor of the invention can be chemically synthesized, for example, by the solid phase peptide synthesis of Merrifield et al (1964). Alternatively, a peptide inhibitor of the invention can be
30 synthesized using standard solution methods (see, for example, Bodanszky, 1984). Newly synthesized peptides can be purified, for example, by high performance liquid

chromatography (HPLC), and can be characterized using, for example, mass spectrometry or amino acid sequence analysis.

The peptide inhibitors of the invention are particularly useful when they are maintained in a constrained secondary conformation. The terms "constrained secondary structure," "stabilized" and "conformationally stabilized" indicate that the peptide bonds comprising the peptide are not able to rotate freely but instead are maintained in a relatively fixed structure. A method for constraining the secondary structure of a newly synthesized linear peptide is to cyclize the peptide using any of various methods well known in the art. For example, a cyclized peptide inhibitor of the invention can be prepared by forming a peptide bond between non-adjacent amino acid residues as described, for example, by Schiller et al (1985). Peptides can be synthesized on the Merrifield resin by assembling the linear peptide chain using N^α-Fmoc-amino acids and Boc and tertiary-butyl proteins. Following the release of the peptide from the resin, a peptide bond can be formed between the amino and carboxy termini.

A newly synthesized linear peptide can also be cyclized by the formation of a bond between reactive amino acid side chains. For example, a peptide containing a cysteine-pair can be synthesized, with a disulfide bridge, can be formed by oxidizing a dilute aqueous solution of the peptide with K₃Fe(CN)₆. Alternatively, a lactam such as an ε-(γ-glutamyl)-lysine bond can be formed between lysine and glutamic acid residues, a lysinonorleucine bond can be formed between lysine and leucine residues or a dityrosine bond can be formed between two tyrosine residues. Cyclic peptides can be constructed to contain, for example, four lysine residues, which can form the heterocyclic structure of desmosine (see, for example, Devlin, 1997). Methods for

forming these and other bonds are well known in the art and are based on well-known rules of chemical reactivity (Morrison et al, 1992).

Alternatively, the peptide inhibitor of the invention can be produced recombinantly. Systems for cloning and expressing polypeptide of the invention include various microorganisms and cells that are well known in recombinant technology. These include, for example, various strains of *E. coli*, *Bacillus*, *Streptomyces*, and *Saccharomyces*, as well as mammalian, yeast and insect cells. The peptide inhibitor of the invention can be produced as a peptide or fusion protein. Suitable vectors for producing the peptide inhibitor are known and available from private and public laboratories and depositories and from commercial vendors. See Sambrook et al, (1989). Recipient cells capable of expressing the gene product are then transfected. The transfected recipient cells are cultured under conditions that permit expression of the recombinant gene products, which are recovered from the culture. Host mammalian cells, such as Chinese Hamster ovary cells (CHO) or COS-1 cells, can be used. These hosts can be used in connection with poxvirus vectors, such as vaccinia or swinepox. Suitable non-pathogenic viruses that can be engineered to carry the synthetic gene into the cells of the host include poxviruses, such as vaccinia, adenovirus, retroviruses and the like. A number of such non-pathogenic viruses are commonly used for human gene therapy, and as carrier for other vaccine agents, and are known and selectable by one of skill in the art. The selection of other suitable host cells and methods for transformation, culture, amplification, screening and product production and purification can be performed by one of skill in the art by

reference to known techniques (see, e.g., Gething et al, 1981).

Pharmaceutical Compositions. The invention provides pharmaceutical compositions comprising a 5 therapeutically effective dose of the peptide inhibitor of the invention in a pharmaceutically acceptable excipient, for administration to a subject, such as a human patient.

"Therapeutically effective amount" as used herein refers to that amount that is effective to obtain the 10 desired therapeutic result. The term "an effective amount" of an inhibitor of GSK3 refers to an amount that is effective to induce an inhibition of GSK3 activity. That activity can be GSK3 kinase activity. The inhibitory amount may be determined directly by measuring the inhibition of a 15 GSK3 activity, or, for example, where the desired effect is an effect on an activity downstream of GSK3 activity in a pathway that includes GSK3, the inhibition may be measured by measuring a downstream effect. Thus, for example where inhibition of GSK3 results in the arrest of phosphorylation 20 of glycogen synthase, the effects of the inhibitor may be effects on an insulin-dependent or insulin-related pathway, and the inhibitor may be administered to the point where glucose uptake is increased to optimal levels. Also, where the inhibition of GSK3 results in the absence of 25 phosphorylation of a protein that is required for further biological activity, for example, the tau protein, then the inhibitor may be administered until polymerization of phosphorylated tau protein is substantially arrested. Therefore, the inhibition of GSK3 activity will depend in 30 part on the nature of the inhibited pathway or process that involves GSK3 activity, and on the effects that inhibition of GSK3 activity has in a given biological context.

The amount of the inhibitor that will constitute an inhibitory amount will vary depending on such parameters as the inhibitor and its potency, the half-life of the inhibitor in the body, the rate of progression of the disease or biological condition being treated, the responsiveness of the condition to the dose of treatment or pattern of administration, the formulation, the attending physician's assessment of the medical situation, and other relevant factors, and in general the health of the patient, and other considerations such as prior administration of other therapeutics, or co-administration of any therapeutic that will have an effect on the inhibitory activity of the inhibitor or that will have an effect on GSK3 activity, or a pathway mediated by GSK3 activity. It is expected that the inhibitory amount will fall in a relatively broad range that can be determined through routine trials.

"Co-administration" as used herein means administration of an inhibitor of GSK3 according to the method of the invention in combination with a second therapeutic agent. The second therapeutic agent can be any therapeutic agent useful for treatment of the patient's condition. For example, inhibition of GSK3 with lithium as a second therapeutic agent used in conjunction with a therapeutic agent inhibitor of GSK3 is contemplated. Additionally, for example, a first therapeutic agent can be a small molecule inhibitor of GSK3 activity, and a second therapeutic agent can be an antisense or ribozyme molecule against GSK3 that, when administered in a viral or nonviral vector, will facilitate a transcriptional inhibition of GSK3 that will complement the inhibitory activity of the small molecule. The second therapeutic agent can also be lithium ion. Co-administration may be simultaneous, for example, by administering a mixture of the therapeutic agents, or may be

accomplished by administration of the agents separately, such as within a short time period. Co-administration also includes successive administration of an inhibitor of GSK3 and one or more of another therapeutic agent. The second

5 therapeutic agent or agents may be administered before or after the inhibitor of GSK3. The second therapeutic agent may also be an inhibitor of GSK3, which has particular advantages when administered with the first inhibitor.

Dosage treatment may be a single dose schedule or a multiple
10 dose schedule.

The term "pharmaceutically acceptable excipient" includes any solvents, dispersion media, antibacterial and antifungal agents, microcapsules, liposomes, cationic lipid carriers, isotonic and absorption delaying agents and the

15 like which are not incompatible with the active ingredients. The formulation of pharmaceutical compositions is generally known in the art; reference can conveniently be made to

Remington's Pharmaceutical Sciences, 18th Ed. (Mack Publishing Co., Easton, PA). Thus, the pharmaceutical

20 compositions can comprise a suitable application medium, such as a gel, salve, lotion, colloid or powder, aqueous solutions such as physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, oils such as olive oil or injectable organic esters. Pharmaceutical

25 compositions can be prepared as injectables, either as liquid solutions or suspensions. The preparation can also be emulsified. The skilled artisan will recognize that any pharmaceutically acceptable means for effecting the introduction of peptide inhibitors into target cells is

30 suitable.

Physiologically acceptable excipients include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or

glutathione, chelating agents, low molecular weight proteins or other stabilizers or excipients. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition,
5 if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents that enhance the effectiveness of the active ingredient. A peptide inhibitor can also be formulated into the pharmaceutical composition as
10 neutralized pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the peptide inhibitor or antibody molecule) that are formed with inorganic acids such as, for example, hydrochloric or
15 phosphoric acids, or such organic acids as acetic, tartaric, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine,
20 trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The pharmaceutical forms suitable for infusion include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile
25 injectable solutions or dispersion. The form is fluid to the extent that easy syringability exists. Typical excipients include a solvent or dispersion medium containing, for example, water-buffered aqueous solutions (i.e., biocompatible buffers), ethanol, polyols, such as
30 glycerol, propylene glycol, polyethylene glycol, suitable mixtures thereof, surfactants, or vegetable oils. Sterilization can be accomplished by any art-recognized technique including, but not limited to, filtration or

addition of antibacterial or antifungal agents, for example, paraben, chlorobutanol, phenol, sorbic acid or thimerosal. Further, isotonic agents, such as sugars or sodium chloride, may be incorporated in the pharmaceutical compositions.

- 5 Prevention against microorganism contamination can be achieved through the addition of various antibacterial and antifungal agents.

Methods of Administration. The peptide inhibitors of the invention can be administered to a subject in any 10 manner known to those of skill in the art which is suitable for the introduction of peptide inhibitor into target cells. For example, the administration of peptide inhibitor blocks about 50% or greater of GSK-3 phosphorylation activity, as measured by *in vitro* kinase assay (see Example 2).

15 The peptide inhibitors of the invention can be administered in any way that is medically acceptable. The mode of administration can depend on the disease condition or injury being treated. Possible administration routes include injections, by parenteral routes, such as 20 intravascular, intravenous, intra-arterial, subcutaneous, intramuscular, intratumor, intraperitoneal, intraventricular, intraepidural or others, as well as oral, nasal, ophthalmic, rectal, topical, or by inhalation.

Sustained release administration is also specifically 25 included in the invention, by such means as depot injections or erodible implants. Administration can also be intra-articularly, intrarectally, intraperitoneally, intramuscularly, subcutaneously, or by aerosol inhalant. Where treatment is systemic due, the composition can be 30 administered orally or parenterally, such as intravenously, intramuscularly, subcutaneously, intraorbitally, intracapsularly, intraperitoneally or intracisternally, as

long as provided in a composition suitable for effecting the introduction of the peptide inhibitor into target cells.

The pharmaceutical compositions can be administered intravenously, as by injection of a unit dose.

- 5 The term "unit dose" when used in reference to a pharmaceutical composition of the invention refers to physically discrete units suitable as unitary dosages for humans, each unit containing a predetermined quantity of active material calculated to produce the desired
- 10 therapeutic effect.

The quantity to be administered depends on the subject to be treated, capacity of the subject to utilize the active ingredient, and degree of inhibition of receptor-ligand binding desired. Precise amounts of active

- 15 ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosage ranges can be one to several mg of active ingredient per individual per day and depend on the route of administration. Suitable regimes for
- 20 initial administration and booster shots are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusion sufficient to maintain therapeutically effective
- 25 concentrations in the blood are contemplated. Whenever, the peptide inhibitors of the invention are used for promoting attachment of cells, such compositions will typically have a higher concentration than those taken internally.

- 30 Dosage. The precise therapeutically-effective amount of peptide inhibitor of the invention used in the methods of this invention applied to humans can be determined by the ordinarily-skilled artisan with consideration of individual differences in age, weight,

extent of cellular infiltration by inflammatory cells and condition of the patient. The pharmaceutical preparation of the invention should be administered to provide an effective concentration of 5-100 μ M, preferably about 5 μ M.

5 The concentration of a peptide inhibitor of the invention required to obtain an effective dose in a subject depends on many factors including the age and general health of the subject as well as the route of administration and the number of treatments to be administered. The total
10 effective amount of a peptide inhibitor of the invention can be administered to a subject as a single dose, either as a bolus or by infusion over a relatively short period of time, or can be administered using a fractionated treatment protocol, in which the multiple doses are administered over
15 a more prolonged period of time.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. "Dosage unit form" refers to physically discrete units suited as unitary
20 dosages for the mammalian subjects to be treated, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical excipient. A pharmaceutical composition typically contains an amount of
25 at least 0.1 weight % of active ingredient, i.e., a peptide inhibitor or antibody of this invention, per weight of total pharmaceutical composition. A weight % is a ratio by weight of active ingredient to total composition. Thus, for example, 0.1 weight % is 0.1 grams of peptide inhibitor per
30 100 grams of total composition.

Methods for Inhibiting the Activity of GSK-3. The peptide inhibitors of the invention are used to inhibit the activity of GSK-3 in a cell. Cells are contacted with a

peptide inhibitor in an amount effective to inhibit GSK-activity *in vitro*. The peptide inhibitors of the invention can be used to potentiate insulin signaling in a subject, *in vivo*. The inhibitor is administered to the subject in an 5 amount effective to increase basal GS activity in intact cells (see Example 3).

Methods for Potentiating Insulin Signaling.

Potentiation of insulin signaling, *in vivo*, resulting from administration of the GSK-3 peptide inhibitors of the 10 invention can be monitored as a clinical endpoint. In principle, the easiest way to look at insulin potentiation in a patient is to perform the glucose tolerance test. After fasting, glucose is given to a patient and the rate of 15 the disappearance of glucose from blood circulation (namely glucose uptake by cells) is measured by assays well known in the art. Slow rate (as compared to healthy subject) of glucose clearance will indicate insulin resistance. The administration of peptide inhibitor to an insulin-resistant patient increases the rate of glucose uptake as compared to 20 a non-treated patient. Peptide inhibitor may be administered to an insulin resistant patient for a longer period of time, and the levels of insulin, glucose, and leptin in blood circulation (which are usually high) may be determined. Decrease in glucose levels will indicate that 25 the peptide inhibitor potentiated insulin action. A decrease in insulin and leptin levels alone may not necessarily indicate potentiation of insulin action, but rather will indicate improvement of the disease condition by other mechanisms.

30 Methods for Treating Diabetes. Diabetes mellitus is a heterogeneous primary disorder of carbohydrate metabolism with multiple etiologic factors that generally involve insulin deficiency or insulin resistance or both.

Type I, juvenile onset, insulin-dependent diabetes mellitus, is present in patients with little or no endogenous insulin secretory capacity. These patients develop extreme hyperglycemia and are entirely dependent on exogenous 5 insulin therapy for immediate survival. Type II, or adult onset, or non-insulin-dependent diabetes mellitus, occurs in patients who retain some endogenous insulin secretory capacity, but the great majority of them are both insulin deficient and insulin resistant. Approximately 95% of all 10 diabetic patients in the United States have non-insulin dependent, Type II diabetes mellitus (NIDDM), and, therefore, this is the form of diabetes that accounts for the great majority of medical problems. Insulin resistance is an underlying characteristic feature of NIDDM and this 15 metabolic defect leads to the diabetic syndrome. Insulin resistance can be due to insufficient insulin receptor expression, reduced insulin-binding affinity, or any abnormality at any step along the insulin signaling pathway (see United States patent 5,861,266).

20 The peptide inhibitors of the invention can be used to therapeutically treat type 2 diabetes in a patient with type 2 diabetes. A therapeutically effective amount of the inhibitor is administered to the patient, and clinical markers, or example blood sugar level, are monitored. The 25 peptide inhibitors of the invention can further be used to prevent type 2 diabetes in a subject. A prophylactically effective amount of the inhibitor is administered to the patient, and a clinical marker, for example IRS-1 phosphorylation, is monitored.

30 Treatment of diabetes is determined by standard medical methods. A goal of diabetes treatment is to bring sugar levels down to as close to normal as is safely possible. Commonly set goals are 80-120 milligrams per

deciliter (mg/dl) before meals and 100-140 mg/dl at bedtime. A particular physician may set different targets for the patient, depending on other factors, such as how often the patient has low blood sugar reactions. Useful medical tests 5 include tests on the patient's blood and urine to determine blood sugar level, tests for glycated hemoglobin level (HbA_{1c}; a measure of average blood glucose levels over the past 2-3 months, normal range being 4-6%), tests for cholesterol and fat levels, and tests for urine protein 10 level. Such tests are standard tests known to those of skill in the art (see, for example, American Diabetes Association, 1998). A successful treatment program can also be determined by having fewer patients in the program with diabetic eye disease, kidney disease, or nerve disease.

15 Methods for Identifying Inhibitors of Insulin

Signaling. The peptide inhibitors of the invention are used to identify inhibitors of GSK-3. Inhibitors of GSK-3 can be identified in a screening assay, using a peptide inhibitor of the invention as a positive control.

20 In one screening method, a first cell is contacted with a test compound that is suspected of interfering with insulin signaling. A second cell with a peptide inhibitor of the invention is contacted with the same test compound. The insulin signal for the first cell is then compared with 25 the insulin signaling for an uncontacted cell and with the insulin signaling for the second cell. A decrease in insulin signaling for the first cell, as compared with the insulin signaling for an uncontacted cell and with the insulin signaling for the second cell, identifies the 30 compound as a compound that interferes with GSK-3 signaling.

The details of one or more embodiments of the invention are set forth in the accompanying description above. Although any methods and materials similar or

equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are now described. Other features, objects, and advantages of the invention will be apparent from the 5 description and from the claims. In the specification and the appended claims, the singular forms include plural referents unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by 10 one of ordinary skill in the art to which this invention belongs. All patents and publications cited in this specification are incorporated by reference.

The following Examples are presented in order to more fully illustrate the preferred embodiments of the 15 invention. These Examples should in no way be construed as limiting the scope of the invention, as defined by the appended claims.

EXAMPLE 1: SYNTHESIS OF A GSK-3 PEPTIDE INHIBITOR

Small, synthetic peptide inhibitors, which are 20 highly specific for GSK-3, were synthesized as described below. The peptides were synthesized according to standard methodologies. Table 1 summarizes the peptides that were synthesized and tested for their ability to inhibit GSK-3. The peptides were patterned after two 25 known substrates of GSK-3, CREB (cAMP response element binding protein) (Fiol et al, 1994), and heat shock factor-1 (HSF-1) (Chu et al, 1996). As illustrated in peptide #1, two serine sites are important in the recognition process. A phosphorylated serine (2) and the 30 serine phosphorylated by the enzyme (1) the two serine are separated by 3 amino acids. This recognition process is also demonstrated in peptide #3 (p9CREB), which is the

same as peptide #1 shortened to 9 amino acids, which peptide is also a good substrate of GSK-3 (see Eldar-Finkelman et al, 1997; and Hallstrom et al, 1998). The presence of phosphorylated serine (site 2) is absolutely required for the enzyme activity. This is demonstrated by a non- phosphorylated CREB peptide (#2) that is not a substrate. Furthermore, replacement of site 2 by glutamic acid which mimics a phosphorylated residue, does not convert this peptide to a substrate (#4). However, replacement of site 1 to alanine converts the peptide to a competitive inhibitor (peptide # 5 see also Fig. 2). Similarly, when site 1 of the phosphopeptide derived from HSF-1 is replaced with alanine (#7), the peptide becomes a competitive inhibitor. Replacement of glutamic acid located downstream of the site 1 of peptide #7 (bold) improves the inhibitory effect of this peptide (#8). Thus, glutamic acid is important for enzyme-substrate/inhibitor interaction. Our data also indicate that at least 4 amino acids downstream of serine site 1 must be present. Otherwise this peptide does not inhibit the enzyme (peptide# 6, 9, 10, 11).

Table 1
Synthetic Peptides Are Listed and Their Kinetic Properties Are Indicated

Peptide #	SEQ ID NO	# Residues	Property
1. KRREILSRRPS(p)YR	1	13	A substrate Derived from CREB protein
2. KRREILSRRPSYR	2	13	Not a substrate
3. ILSRPS(p)YR	3	9	A substrate
4. ILSRPEYR	4	9	Neither a substrate nor an inhibitor
5. KRREILARRPS(p)YR	5	13	An inhibitor IC ₅₀ =330 μM
6. EILARRPS(p)Y	6	9	Does not inhibit
7. KEEPPAPPQS(p)P	7	11	(patterned after HSF-1 protein) An inhibitor (IC ₅₀ =250 μM)
8. KEAPPAPPQS(p)P	8	11	An inhibitor (IC ₅₀ =190 μM)
9. EPPARRE	9	7	Does not inhibit
10. EPPAPR	10	6	Does not inhibit
11. PAPPQS(p)P	11	7	Does not inhibit

EXAMPLE 2: SELECTIVE INHIBITION OF GSK-3 BY A PEPTIDE INHIBITOR

Inhibition of Substrate Phosphorylation Activity

5 of GSK-3. The inhibition of GSK-3 activity by a small, synthetic peptide inhibitor synthesized as described above in Example 1, was tested by *in vitro* kinase assay. Peptide inhibitor #5 (see Table 1) is a 13 amino acid synthetic peptide having the sequence KRREILARPS(p)YR (SEQ ID NO:5, where S(p) = phosphoserine, and A represents the Ser to Ala substitution at the GSK-3 phosphorylation site.

10 Inhibition of GSK-3 by the peptide inhibitor #5 was determined by performing *in vitro* kinase assays of GSK-3 with a peptide substrate and determination of ³²P incorporation into the substrate. *In vitro* kinase assays were performed utilizing purified recombinant rabbit GSK-3 using the method of Eldar-Finkelman et al (1997), a peptide substrate PGS-1 peptide (see Hallstrom et al, 1998), various concentrations of the peptide inhibitor, and ³²P_γ-ATP. The

assay mixture contained: 0.5 μ l GSK-3 (0.5 μ g), 50 mM Tris, 10 mM MgAc, 0.01% β -mercaptoethanol, 50 μ M 32 P γ -ATP (0.25 μ Ci/assay), and 100 μ M PSG-1 peptide. Reaction mixtures were incubated at 30°C for 15 minutes, then spotted on p81 phosphocellulose paper squares, washed with 100 mM phosphoric acid, dried and counted for radioactivity.

The results of this representative *in vitro* inhibition assay of GSK-3 with an exemplary peptide inhibitor, peptide #5, are shown in Figure 1. These results show the strong inhibition of GSK-3 phosphorylation of its substrate by the peptide inhibitor ($IC_{50}=150 \mu M$). Similar results were obtained when a different peptide substrate, PGS-1 (a peptide patterned after glycogen synthase), was used.

The inhibition of GSK-3 by peptides #5, 7 and 8 is shown in Figure 2. Lineweaver-Burk plots revealed that all three enzymes are competitive inhibitors. The data also suggest that peptide #8 is the best inhibitor. K_i values ranged from 30-80 μM .

EXAMPLE 3: The Effect of Peptide Inhibitor on Glycogen Synthase in Intact Cells

The present inventor examined whether the peptide inhibitors are capable of inhibiting GSK-3 activity in intact cells. There is a substantial evidence that insulin-activation of glycogen synthase (GS) is via inhibition of GSK-3. Peptide #5 was, therefore, tested to see if it enhanced GS activity in intact cells. In order to improve the delivery of the peptide into cells, synthesized peptide #5 was linked to a hydrophobic cell permeable peptide (16 amino acids (Hawiger 1997)), termed here HP9c. 293 cells were treated with increased concentrations of HP9c, and GS activity was measured as

previously described (Eldar-Finkelman et al, 1996). Treatment of cells with the peptides resulted in increased basal GS activity. The effective peptide concentrations ranged between 60-100 μ M (Figure 3).

5 Phosphorylation of GS by GSK-3 also enhances GS sensitivity to its allosteric regulator glucose 6 phosphate (G6P). This effect was shown *in vitro* and in intact cells (L.M. Carmody, B. Plotkin, H. Eldar-Finkelman. "The insulin-like action of lithium in human
10 cultured cells" submitted). Thus, if GSK-3 is inhibited in intact cells, then activation of GS by G6P should enhance. Figure 4 shows that treatment of cells with HP9c peptide inhibitor resulted in enhanced activation of GS by G6P. In this experiments G6P dose response activation of
15 GS that was isolated from peptide-treated and non-treated cells was examined. Results show that GS from treated cells (hollow circles) is better activated by G6P as compared to control.

EXAMPLE 4: Treating a Patient with NIDDM

20 A patient is diagnosed in the early stages of non-insulin dependent diabetes mellitus. A peptide inhibitor of GSK-3 in accordance with the present invention is formulated in an enteric capsule. The patient is directed to take one tablet after each meal for the purpose of stimulating the
25 insulin signaling pathway, and thereby controlling glucose metabolism to levels that obviate the need for administration of exogenous insulin.

EXAMPLE 5: Treating a Patient with Alzheimer's Disease

A patient is diagnosed with Alzheimer's disease.
30 The patient is administered a selective peptide inhibitor of the present invention, which inhibits GSK-3-mediated tau

hyperphosphorylation prepared in a formulation that crosses the blood/brain barrier. The patient is monitored for tau phosphorylated polymers by periodic analysis of proteins isolated from the patient's brain cells for the presence of 5 phosphorylated forms of tau on an SDS-PAGE gel known to characterize the presence of and progression of the disease. The dosage of the inhibitor is adjusted as necessary to reduce the presence of the phosphorylated forms of tau protein.

10 The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without undue experimentation and
15 without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed
20 herein is for the purpose of description and not of limitation. The means, materials, and steps for carrying out various disclosed functions may take a variety of alternative forms without departing from the invention.
Thus the expressions "means to..." and "means for...", or
25 any method step language, as may be found in the specification above and/or in the claims below, followed by a functional statement, are intended to define and cover whatever structural, physical, chemical or electrical element or structure, or whatever method step, which may now
30 or in the future exist which carries out the recited function, whether or not precisely equivalent to the embodiment or embodiments disclosed in the specification above, i.e., other means or steps for carrying out the same

functions can be used; and it is intended that such expressions be given their broadest interpretation.

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WHAT IS CLAIMED IS:

1. A peptide inhibitor of glycogen synthase kinase-3 (GSK-3), wherein the inhibitor comprises a polypeptide from 7 to 50 amino acids having the amino acid sequence XZXXXS(p)X, wherein S(p)=phosphorylated serine or threonine, wherein X=any amino acid, and wherein Z=any amino acid except serine or threonine, and having the ability to inhibit the enzymatic activity of GSK-3, with the proviso that the polypeptide does not contain two or more SXXXS motifs, wherein S=serine, upstream of the S(p) residue.

2. A peptide inhibitor in accordance with claim 1, wherein said polypeptide has a length of from 7 to 20 amino acids.

3. A peptide inhibitor in accordance with claim 1, wherein said polypeptide has a length of from 10 to 13 amino acids.

4. A peptide inhibitor in accordance with claim 1, wherein said polypeptide is a fragment of a GSK-3 substrate containing a single SXXXS recognition motif in which the upstream S of the recognition motif is replaced by any amino acid other than serine or threonine and the downstream S is phosphorylated.

5. A peptide inhibitor in accordance with claim 4, wherein the said substrate is the CREB protein.

6. A peptide inhibitor in accordance with claim 4, wherein the said substrate is the HSF-1 protein.

7. A peptide inhibitor in accordance with claim 1 having at least three amino acid residues upstream of the Z amino acid residue and where the residue at the position three residues upstream of Z is any amino acid other than glutamic acid.

8. A method for inhibiting the phosphorylation activity of GSK-3, comprising contacting a cell with a

peptide inhibitor in accordance with claim 1 in an amount effective to inhibit GSK-3 phosphorylation activity.

9. A method of treating a biological condition mediated by GSK-3 activity, comprising administering an effective amount of a peptide inhibitor in accordance with claim 1.

10 10. A method in accordance with claim 9, wherein said biological condition is non-insulin dependent diabetes mellitus.

11. A method in accordance with claim 9, wherein said biological condition is non-insulin dependent Alzheimer's Disease.

12. A method in accordance with claim 9, wherein said biological condition is non-insulin dependent manic depression.

13. A method for preventing non-insulin dependent mellitus, comprising administering a prophylactically effective amount of a peptide inhibitor in accordance with claim 1 to a subject.

20 14. A pharmaceutical composition comprising the peptide inhibitor of claim 1 in a pharmaceutically acceptable excipient.

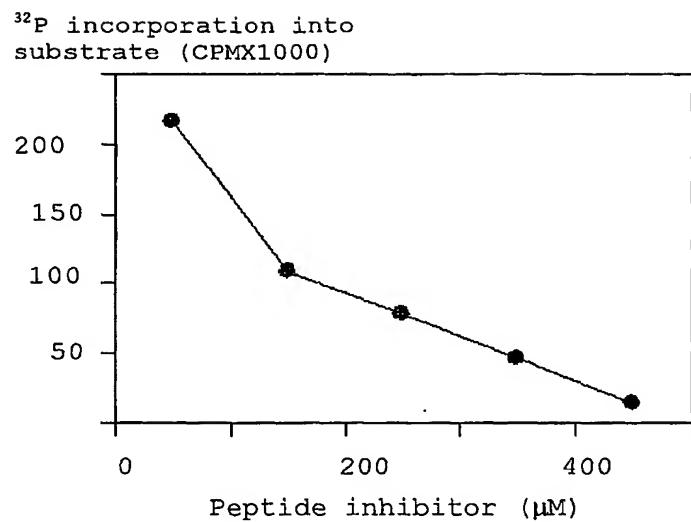
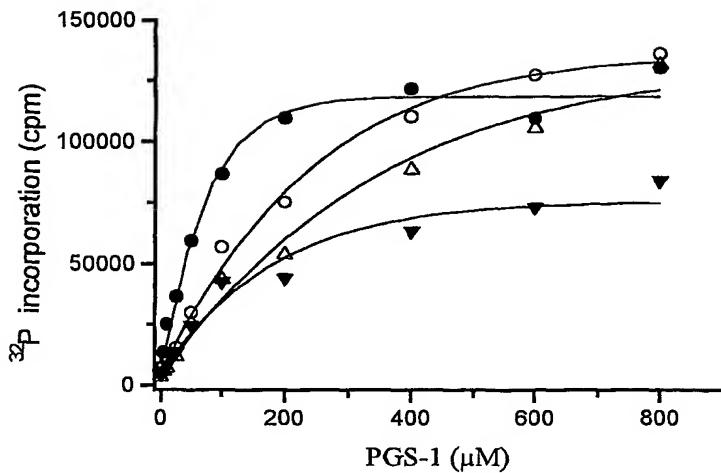
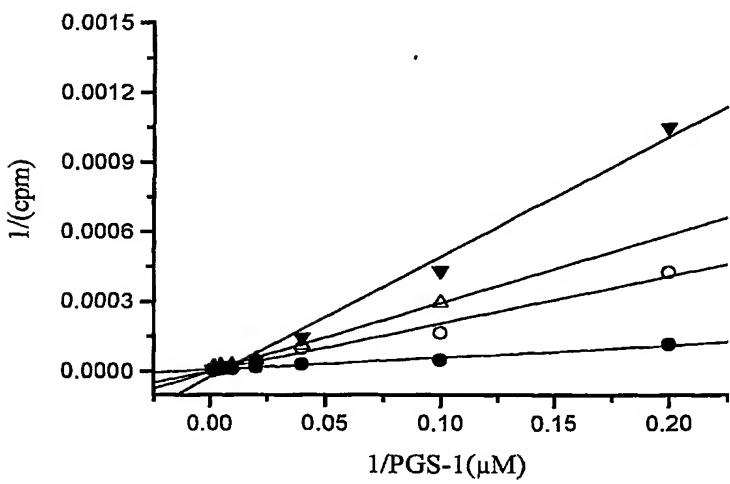
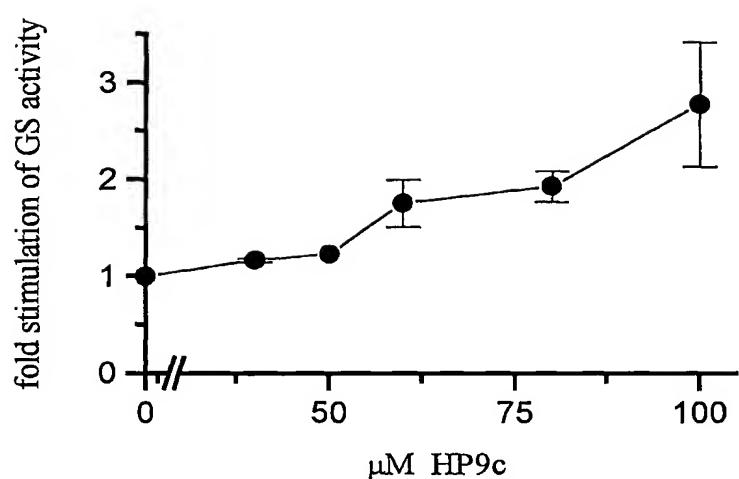
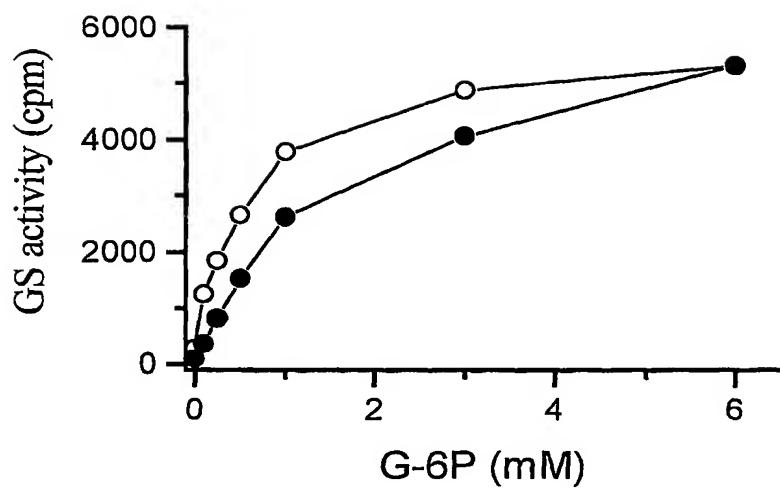


FIGURE 1

**FIGURE 2A****FIGURE 2B**

**FIGURE 3**

**FIGURE 4**

INTERNATIONAL SEARCH REPORT

Int. Application No
PCT/US 01/00123

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K/06 C12N9/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	C.DUTRA ET AL.: "Unique Toxic Peptides Isolated from Sawfly Larvae in three Continents" TOXICON, vol. 37, no. 3, 1999, pages 537-44, XP000994829 figure 3 ---	1-4, 7, 14 -/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

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Date of the actual completion of the International search	Date of mailing of the International search report
5 April 2001	12/04/2001
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Deffner, C-A

INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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